

**IMMUNE PRIMING**  
Immunize mice with imported  
fire ant midgut tissue

**RNA → cDNA**  
Isolate total RNA from spleen of immunized mice,  
prepare cDNA by reverse transcription, amplify  
by polymerase chain reaction, purify cDNA from gel

**ANTIBODY LIBRARY ON  
SURFACE OF PHAGE**  
Create phage display library  
expressing  $10^6$ - $10^8$  unique  
antibody Fab fragments

**DUAL MIDGUT SELECTION**  
Two-step absorptions to yield  
phage displaying antibody fragments  
specific for midgut of imported fire ants  
and not native fire ants

**FINAL MICROVILLI SELECTION**  
Immunohistochemical verification of  
Fab specific to imported fire ant  
microvilli cells

**TESTING**  
Test phage/Fab for internalization by  
microvilli cells of imported fire ants  
when administered by feeding

**IMPORTED FIRE ANT ERADICATION**  
Test phage/Fab/gelonin Conjugate for ability  
to selectively kill imported fire ants.

**FIGURE 1**

Figure 2 Evaluation of Monoclonal Antibodies to Midgut Antigens of Imported and Native Fire Ant queens.

Immunohistological analyses of monoclonal antibody binding to the midgut antigens of imported fire ant queens (Positive, B) but not to the midgut antigens of midgut antigens from native fire ants (Native, C). Midgut antigens from imported fire ants reacted with irrelevant antibody did not stain positive and served as the negative control (Control, A)

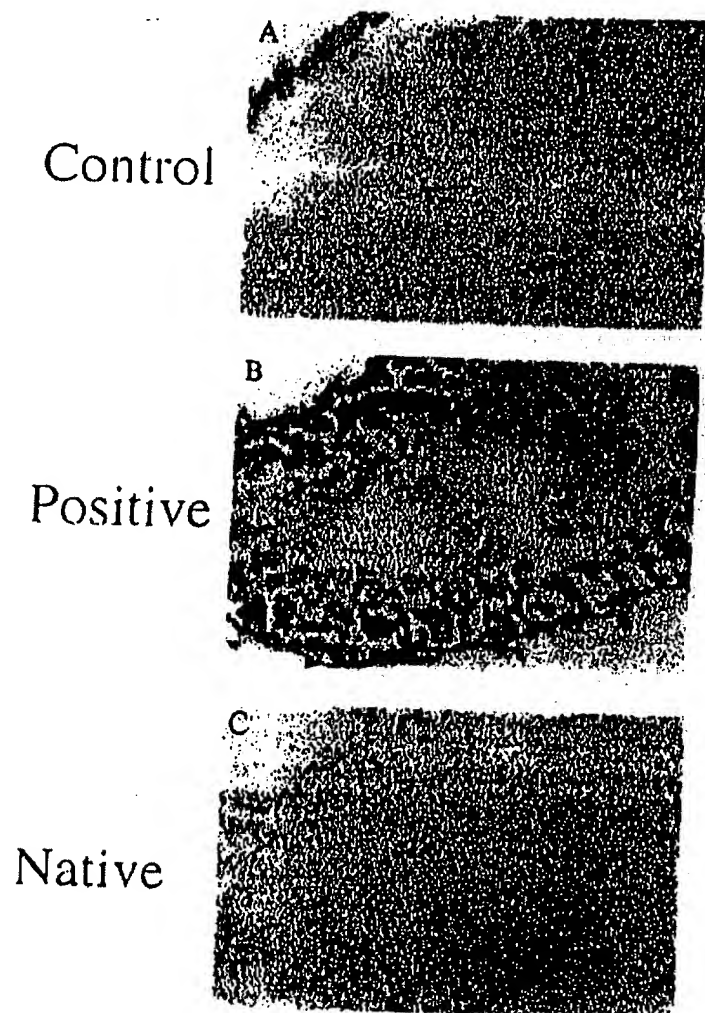


Fig. 2

Figure 2, Purification of Phage Displayed Fab to Midgut Antigens of Imported Fire Ant Queens.

# EVIDENCE OF PRESENCE OF IG FAB FRAGMENT

C = Control

1-4 = eluted soluble Fab (sFab)

46 = size of Fab fragment (46 Kb)

Western immunoblot analyses show the clone to express Ig Fab. These clones were selected for ability to bind to antigens of the midgut of imported fire ants but not to the midgut antigens of native fire ants.

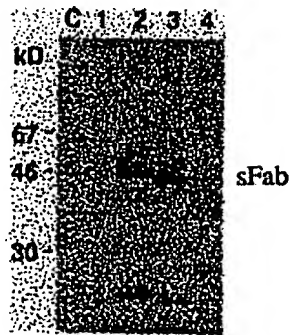


Fig. 3

Figure 4. Purification of Phage Displayed Fab to Gelonin

EVIDENCE OF PRESENCE OF IG FAB FRAGMENT FOR TWO CLONES: (pComb3/Fab (6) and pComb3/Fab (47).

IN = Induced

U = Uninduced

V = bacteria containing virus without Ig Fab

46 Kb = size of Fab fragment

Western immunoblot analyses show that clones pComb3/Fab (6) and pComb3/Fab (47) express Ig Fab. These clones were selected for ability to bind to gelonin.

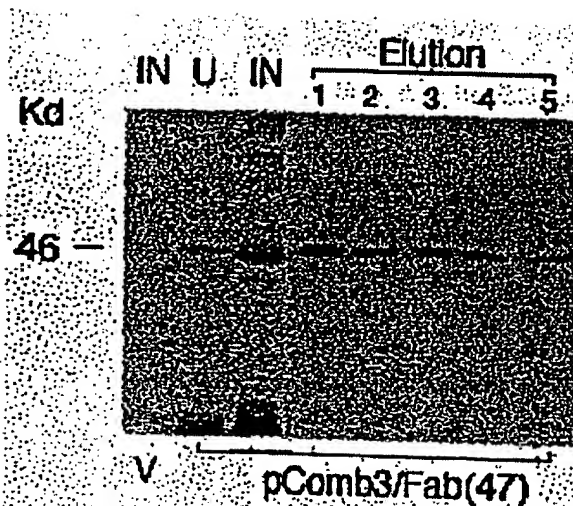
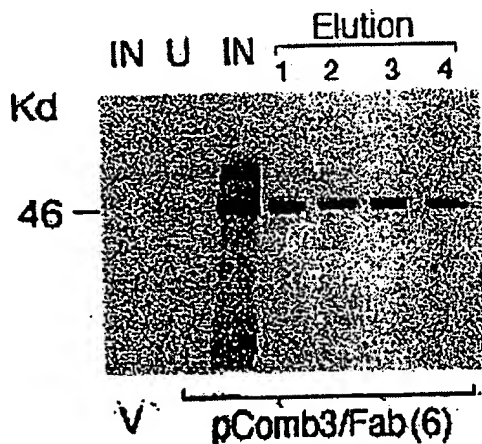


Fig. 4